mutation in ORAI1 C-terminus abrogated communication with STIM1 C-terminus, while an analogous mutation in ORAI2 and ORAI3 still allowed for their moderate activation. Conversely, destabilizing the second coiled-domain of STIM1 C-terminus by a single point mutation still enabled partial stimulation of ORAI2 and ORAI3 channels but not of ORAI1. A double mutation within the second coiled-coil motif of STIM1 C-terminus fully disrupted communication with all three ORAI channels. In aggregate, the impairment in the overall communication between STIM1 and ORAI channels upon mutual destabilization of putative coiled-coil domains in either C-terminus would be compatible with their heteromeric interaction.Supported by FWF P18169.

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An Orai1 Activating Minimal Fragment Of Stim1

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In immune cells generation of sustained Ca2+ levels is mediated by the Ca2+ release activated Ca2+ (CRAC) current. Molecular key players in this process comprise the stromal interaction molecule (STIM1) that functions as a Ca2+ sensor in the endoplasmic reticulum and ORAI1 located in the plasma membrane. Depletion of ER Ca2+ store leads to STIM1 multimerization into discrete punctae that co-cluster with ORAI1 thereby triggering coupling to and activation of ORAI1 channels. The C-terminus of STIM1 is sufficient for the activation of ORAI1 currents independent of store depletion. Here we unmasked an ORAI activating minimal fragment (OAMF) within STIM1 C-terminus that exhibited enhanced interaction with ORAI1 and resulted in threefold increased Ca2+ currents. STIM1-OAMF still showed the ability of a homomeric interaction similar to longer fragments as well as the full-length form of STIM1 C-terminus. In contrast, further deletion of a thirty amino acid region resulted in a substantial reduction of homomeric interaction concomitant to a loss of coupling to as well as activation of ORAI1. In aggregate, we have identified two key regions within STIM C-terminus that govern ORAI1 activation. (Supported by PhD-Program W1201 from the FWF)

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Increased Hydrophobicity At The N-terminus/membrane Interface Impairs Gating Of The Scid-related Orai1 Mutant

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Patients with severe combined immune deficiency (SCID) suffer from defective T cell Ca2+ signalling. At the molecular level a loss of Ca2+ entry has been linked to a single missense mutation R91W in the store-operated Ca2+ channel Orai1. Yet, the mechanistic impact of this mutation on Orai1 function remains unclear. Confocal FRET microscopy revealed that dynamic store-operated STIM1 coupling to Orai1 R91W was preserved similar to wild-type Orai1. Characterization of various point mutants at position 91 by whole-cell patch-clamp recordings revealed that neutral or even negatively charged amino acids did not impair Orai1 function. However, a substitution by hydrophobic leucine, valine or phenylalanine resulted in non-functional Orai1 channels. Bioinformatic analysis on secondary structure of the ASSR moiety (amino acid 88–91) that is located at the N-terminus/membrane interface suggested conformational constraints when R is substituted by these hydrophobic amino acids. Glycines substituting for the two serines in the ASSR moiety further promoted conformational flexibility and indeed increased channel activity. However, function of the Orai1 R91W mutant was not restored by these two additional glycine substitutions, pointing to a dominant role of tryptophan 91. Transmembrane probability plots revealed a substantial increase in probability for the first transmembrane segment in the case of all the hydrophobic, non-functional Orai1 R91X mutants in contrast to functional ones. We suggest that a substantial increase in the transmembrane probability of the first sequence of Orai proteins together with structural constraints at the N-terminus/membrane interface yields non-functional Orai1 channels. (supported by FWF 18169)

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Structural dynamics of CaMKII activation

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The ubiquitously expressed calcium/calmodulin dependent protein kinase II (CaMKII) functions as a transducer of calcium (Ca^{2+}) signaling by responding to the amplitude, duration, and frequency of Ca^{2+} transients. During periods of elevated Ca^{2+}, CaMKII is activated by calcium-calmodulin (Ca^{2+}/CaM) binding. A subsequent autophosphorylation at Thr286 allows for Ca^{2+}-independent activity and endows this enzyme with a conformational memory of prior activation. CaMKII activity is regulated by a myriad of factors including CaM binding, autophosphorylation, and catalytic-regulatory domain interactions referred to as autoinhibition. While these variables have been linked to CaMKII function, the underlying structural and dynamic framework of activation and conformational memory is poorly understood. Here we utilize site-directed spin labeling and electron paramagnetic resonance (SDSL-EPR) to explore the conformational changes associated with CaMKII activation and conformational memory. EPR parameters were collected for the regulatory domain where CaM binding and autophosphorylation sites are located. Our results indicate the regulatory domain undergoes significant structural changes between several discrete conformations dependent on autophosphorylation and CaM binding. The CaM binding region is flexible in the apo state but has an induced rigidity in the presence of Ca^{2+}/CaM indicative of a binding event. Investigation of the regulatory domain outside the CaM binding region revealed an increase in protein backbone dynamics with a Thr286Glu autophosphorylation mimic and/or in the presence of Ca^{2+}/CaM. This data provides a structural and dynamic perspective consistent with the current biochemical activation model where CaM binding disrupts autoinhibition by disengaging regulatory and catalytic domains. We predict the enhanced flexibility facilitates Ca^{2+}/CaM binding and may play a role in Ca^{2+} independent activity. The adjacent regulatory loop showed similar flexibility suggesting this region functions as a hinge between regulatory and catalytic domains allowing for release and reinstatement of autoinhibition.

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Calcium binding and conformational properties of calmodulin complexed With PEP-19

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PEP-19 is an IQ calmodulin (CaM) binding motif that inhibits apoptosis and protects cells against Ca^{2+} toxicity. We showed that PEP-19 interacts predominantly with the C-domain of CaM, and that it greatly increases the k_{on} and k_{off} rates of Ca^{2+} binding, but has little effect on K_{Ca}. Here we used solution NMR to characterize the calcium binding and conformational properties of the Ca^{2+}-CaM-PEP-19 complex. Both $^{3}J_{HNHA}$ and ^{1}H, ^{15}N NOESY-HSQC experiments show the overall secondary structure of Ca^{2+}-CaM is not greatly affected upon binding PEP-19. ^{15}N backbone dynamics suggests that the Ca^{2+}-CaM-PEP-19 complex shows large-scale dynamics. Most residues in the C domain of CaM that experience significant chemical exchange on μs to ms timescale form a hydrophobic patch to interact with PEP-19.
We used a C-term fragment of CaM, CaM(76–148), which binds two Ca^{2+} ions, to determine the effect of PEP-19 on cooperative Ca^{2+} binding. Highly cooperative Ca^{2+} binding was seen in the absence of PEP-19, giving two sets of peaks in the ^{1}H-^{15}N HSQC spectra at substoichiometric levels of Ca^{2+}, corresponding to apo and 2-Ca^{2+} bound forms of CaM(76–148). However, in the presence of PEP-19, cooperativity was largely lost and most residues in CaM(76–148) showed line broadening, and spitting into multiple peaks at low Ca^{2+} levels. Amide markers in the Ca^{2+} binding loops showed sequential Ca^{2+}-binding first to site IV and then to site III. Furthermore, $^{1}H_{\alpha}$, $^{13}C_{\alpha}$ chemical shift perturbations indicate that the β-strand in Ca^{2+} binding loop III shifts toward the random coil direction in the presence of PEP-19. This indicates that loss of cooperativity and increased in k_{off} and k_{on} rates induced by PEP-19 is caused by destabilizing the antiparallel β-sheet formed between Ca^{2+} binding sites III and IV in the C-domain of CaM.

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Characterization of Calmodulin with Mutated Ca2+-Binding Sites

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Calmodulin (CaM) regulates cellular functions via its Ca2+ binding properties. The N- and C-domains of CaM, which are separated by a flexible tether, each bind two Ca2+ ions via EF-hand motifs. Mutation of position 1 in individual EF hands (the X coordination site) from Asp to Ala has been used to selectively inhibit Ca2+ binding to the N- and C-domains of CaM. We used this mutation strategy to investigate how the individual Ca2+ binding sites contribute to the association of PEP-19 with CaM. Four CaM mutants were made and designated CaM12, CaM3, CaM4 and CaM34 based on nomenclature established in the literature. Ideally, all mutant proteins should be structurally and functionally identical to native CaM in the absence of Ca2+, however, mutation of Ca2+ binding sites in the C-domain of CaM caused weak affinity and significantly different koff and kon rates for binding PEP-19. This led us to use NMR and